isoforms predominantly localized in the mitochondria and were absent or minimally present in the sarcoplasmic reticulum, whereas others abundantly distributed in both cellular compartments. Additionally, select HAX-1 proteins enhanced cell survival following exposure to H2O2, similar to the prototypical variant I, while others interestingly promoted cell death. To better understand the functions of the different HAX-1 isoforms in modulating cell survival and death, current experiments are under way to examine by quantitative RT-PCR their expression profile during homeostasis, after stress induction, and in disease conditions.

212-Pos Board B12
Nogo-A Knockdown Inhibits Hypoxia/Reoxygenation-Induced Activation of Mitochondrial-Dependent Apoptosis in Cardiomyocytes
Jason P. Sarkey.
Nogo-A has been well-characterized as a potent inhibitor of axonal regeneration and plasticity in the central nervous system, however the role of Nogo-A in non-nervous tissues is essentially unknown. In this study, Nogo-A expression was shown to be significantly increased in left ventricular tissue from human patients with DCM and from patients who have experienced an ischemic event. Nogo-A expression was clearly associated with cardiomyocytes in culture and was localized predominantly in the endoplasmic reticulum. In agreement with the findings from human tissue, Nogo-A expression was significantly increased in cultured cardiomyocytes subjected to hypoxia/reoxygenation. Knockdown of Nogo-A in cardiomyocytes markedly attenuated hypoxia/reoxygenation-induced apoptosis, as indicated by the significant reduction of DNA fragmentation, phosphatidylserine translocation, and caspase-3 cleavage, by a mechanism involving the preservation of mitochondrial membrane potential, the inhibition of ROS accumulation, and the inhibition of cytochrome c release. Together, these data indicate that knockdown of Nogo-A in cardiomyocytes may serve as a novel therapeutic strategy in the treatment of ischemic/hypoxic injury.

213-Pos Board B13
Real-Time Dynamics of Ca^{2+}, Phosphatidylserine, Caspase-3/7, and Morphological Changes in Apoptosis: Retinal Ganglion Cells Under Elevated Pressure
Siyuan Lu, Jae Kyoo Lee, Anupam Madhukar.
Quantitative information on the dynamics of multiple molecular processes in individual live cells under controlled stress is central to the understanding of the cell behavior of interest and the establishment of reliable models. We report on the dynamics of the apoptosis regulator intracellular Ca^{2+}, surface marker phosphatidylserine (PS), effector caspase-3/7, and morphological changes examined simultaneously in individual transformed retinal gangling cells undergoing apoptosis at elevated hydrostatic pressure. A custom-designed imaging platform that allows long-term real-time imaging of morphological and molecular-level physiological changes in large numbers of live cells (beyond the field-of-view of typical microscopy) under controlled pressure is employed.[1] Intracellular Ca^{2+} elevation and PS translocation to the outer leaflet of the plasma membrane at the early stages (typically <5 hours after the onset of 100 mmHg pressure) followed by gradual caspase-3/7 activation at late stages (typically >5 hours) is found. The data reveal a strong temporal correlation between the Ca^{2+} elevation, PS translocation, and morphological changes (neurite retraction and soma shrinkage) in the vast majority of the cells. This suggests that Ca^{2+} is likely responsible for the onset of PS translocation and apoptotic morphological changes. Moreover, the data show a significant cell-to-cell variation in the onset of caspase-3/7 activation, an inevitable consequence of the stochastic nature of the underlying biochemical reactions not captured by conventional assays based on population-averaged cellular responses. This study demonstrates that the approach of simultaneously imaging multiple intracellular events in large numbers of live cells provides statistically significant data to enable refinements and testing of models of signaling pathways, here apoptosis. [1] Lee JK, Lu S, Madhukar A (2010) Real-time Dynamics of Ca^{2+}, Caspase-3/7, and Morphological Changes in Retinal Ganglion Cell Apoptosis under Elevated Pressure. *PLoS ONE*, In Press.

214-Pos Board B14
Biophysical Basis for Specificity of Action of Human Isoforms of Secretory Phospholipase A_2 During Cell Death
Jennifer Nelson, Amanda M. Berbert, Elizabeth Gibbons, Katalyn R. Pickett, Michael Streeter, Ashley O. Warcup, Celestine H.-Y. Yeung, Allan M. Judd, John D. Bell.
The hydrolytic activity of secretory phospholipase A_2 ($sPLA_2$) toward mammalian cells depends on the health of the cell. During the process of programmed cell death, changes occur in the cell membrane that render it susceptible to hydrolytic attack. Various isozymes of $sPLA_2$ respond differently to cell death depending on the timing and origin of the process. This study used flow cytometry to classify subpopulations of S49 lymphoma cells during several modes of programmed death depending on their vulnerability to $sPLA_2$ and identify the basis for isozyme specificity. Most death stimuli (thapsigargin, dexamethasone, actinomycin D, paclitaxel, and methotrexate) caused a reduction in membrane lipid-neighbor interactions detected as increased binding of the fluorescent dye merocyanine 540 and modest permeability to a vital stain, propidium iodide. In each of these cases, all $sPLA_2$ isozymes tested (snake venom and human groups IIa, V, and X) displayed enhanced ability to hydrolyze the cell membrane. In contrast, cells exposed to a calcium ionophore showed the increase in merocyanine 540 binding without accompanying permeability to propidium iodide. Under these conditions, only the snake venom and human group X enzymes hydrolyzed cells that were dying. Lastly, the human group IIa enzyme, although most active of the isoforms tested toward anionic artificial bilayers, hydrolyzed dying cells at a rate that was only 1/100 that of the other isozymes. These results suggested that each of these human isozymes fills different physiological roles in responding to cell death and provides probable molecular explanations for the distinctions.

Mitochondria in Cell Life and Death

215-Pos Board B15
Interaction of Creatine Kinase and Nucleoside Diphosphate Kinase with Mitochondrial Cardiolipin Membranes: Differences in Mechanism and in the Effect on Enzyme Catalysis
Malgorzata Tokarska-Schlattner, Marie-Lise Lacombe, **Uwe Schlattner**.
Mitochondrial isoforms of creatine kinase (MtCK) and nucleoside diphosphate kinase (NDPK-D) have critical functions in bioenergetics, membrane topology and organelle morphology with roles in human health and disease. X-ray structural analysis, electron microscopy, surface plasmon resonance (SPR) and scanning calorimetry revealed that both kinases form large oligomers that bind to and cross-link mitochondrial membranes via anionic phospholipids, mainly cardiolipin; at least MtCK can also induce cardiolipin-rich membrane domains. First we used surface plasmon resonance combined with thermodynamic analysis to study kinase/cardiolipin interaction. The two kinases differed in their membrane binding mechanism: (i) NDPK-D showed monophasic binding due to electrostatic interactions of a triad of basic amino acids, while binding of MtCK was biphasic, with only the main component depending on electrostatic interactions of C-terminal basic amino acids. (ii) Rising temperature increased cardiolipin affinity of MtCK, in particular in the second binding component, but not of NDPK-D, indicating hydrophobic interactions in case of MtCK. (iv) Kinase/membrane interaction occurred to be an entropy-driven binding process, in particular for MtCK, possibly due to charge neutralization, release of bound water, and effects on membrane order. Second, we studied the effect of membrane-association on kinase enzyme activity. While basic cardiolipin had no effect on MtCK, NDPK-D was strongly inhibited. This inhibition was relieved by doxorubicin that strongly competes for cardiolipin binding. We propose a model for MtCK and NDPK-D interaction with cardiolipin-containing lipid membranes. For NDPK-D, a single phase, purely electrostatic binding would lead to a partial shielding of the enzymes' active sites and thus catalytic inhibition. For MtCK, a two-phase binding model of rapid electrostatic docking and slower anchoring via hydrophobic stretches is proposed, which does not affect the active sites.

216-Pos Board B16
Probing VDAC Voltage-Gating Mechanism by pH: Functional and Structural Implications
Oscar Teijido Hermida, Matthew T. Eddy, Robert G. Griffin, Sergey M. Bezrukov, Tatiana K. Rostovtseva.
VDAC controls fluxes of ATP/ADP and other respiratory substrates across mitochondrial outer membrane by using its characteristic ability to switch or "gate" between the so-called "open" and "closed" states. While most metabolites go freely through a unique open state, the closed states are virtually impermeable to ATP and ADP. Therefore, unveiling molecular mechanisms of VDAC gating is important in our understanding of mitochondrial respiration and metabolism in health and pathology. Available crystal structure of VDAC solved to the atomic level of resolution does not provide data on VDAC gating mechanism in spite of a number of different models that have been proposed. Although effects of pH on VDAC gating have been shown previously, here we further explore this approach by performing functional and structural studies on VDAC at extremely low pH. In our experiments with VDAC reconstituted into planar lipid membranes, voltage-gating is drastically increased as pH decreases from 7.4 to 3.0. Interestingly, the effect of pH on gating is fully reversible, i.e., gating returns to the initial behavior after returning